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Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach

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Abstract

The soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), is a pest of soybeans in Asia, and in recent years has caused extensive damage to soybeans in North America. Within these agroecosystems, generalist predators form an important component of the assemblage of natural enemies, and can exert significant pressure on prey populations. These food webs are complex and molecular gut-content analyses offer nondisruptive approaches for examining trophic linkages in the field. We describe the development of a molecular detection system to examine the feeding behaviour of *Orius insidiosus* (Hemiptera: Anthocoridae) upon soybean aphids, an alternative prey item, *Neohydatothrips variabilis* (Thysanoptera: Thripidae), and an intraguild prey species, *Harmonia axyridis* (Coleoptera: Coccinellidae). Specific primer pairs were designed to target prey and were used to examine key trophic connections within this soybean food web. In total, 32% of *O. insidiosus* were found to have preyed upon *A. glycines*, but disproportionately high consumption occurred early in the season, when aphid densities were low. The intensity of early season predation indicates that *O. insidiosus* are important biological control agents of *A. glycines*, although data suggest that *N. variabilis* constitute a significant proportion of the diet of these generalist predators. No *Orius* were found to contain DNA of *H. axyridis*, suggesting intraguild predation upon these important late-season predators during 2005 was low. In their entirety, these results implicate *O. insidiosus* as a valuable natural enemy of *A. glycines* in this soybean agroecosystem.

Keywords: *Aphis glycines*, generalist predators, gut-content analysis, *Neohydatothrips variabilis*, PCR, predator–prey interactions

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Introduction

Generalist predator communities are widely acknowledged as providing valuable levels of regulation of pests, such as aphids, in agroecosystems throughout the world (Sunderland *et al*. 1997; Symondson *et al*. 2002). Not only do these natural enemies feed on aphid prey when their densities are very low early in the season (Harwood *et al*. 2004), but their 'lying-in-wait' behaviour (Settle *et al*. 1996) allows them to impact upon target pests immediately after colonization, thereby contributing to valuable levels of control (Fleming 1980). Therefore, through the utilization of alternative prey resources, generalist predators can increase their density early in the season (Butler & O'Neil 2007), before pests arrive, and later switch to feeding on pests (Edwards *et al*. 1979; Chiverton 1987; Settle *et al*. 1996). However, many authors have emphasized that the presence of alternative foods can affect biological control through a variety of mechanisms (Andow & Risch 1985; Musser & Shelton 2003; Prasad & Snyder 2006). For

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example, the presence of one prey species can have negative effects on the population of another prey (e.g. a pest) species by allowing the population of a shared predator to increase, thus leading to higher predation rates upon both prey items (Holt 1977; van Veen *et al*. 2006). In contrast, alternative prey can also lower predation on focal prey because of predator preference for alternative prey resources (Abrams & Matsuda 1996; Harwood *et al*. 2004; Prasad & Snyder 2006). In such instances, the alternative prey can have a positive effect on population densities of focal prey.

In North America, the soybean aphid *Aphis glycines* Matsumura (Homoptera: Aphididae) is a new exotic pest of soybeans. Since the first records documenting its existence in the summer of 2000, these aphids have spread to over 20 states and provinces in the USA and eastern Canada (Ragsdale *et al*. 2004; Venette & Ragsdale 2004). In the US Midwest, the aphid has triggered insecticide applications in soybean fields; in many areas, this was the first time soybean was treated for any insect pest (Rodas & O'Neil 2006). Given the potential for significant yield losses, considerable attention has focused on how natural enemies impact soybean aphid dynamics and damage (Heimpel *et al.* 2004). The complex of predators in North American soybeans is diverse (Rutledge *et al*. 2004), and among these predators, the anthocorid *Orius insidiosus* (Say) (Hemiptera: Anthocoridiae) is a dominant component of the community in Indiana, particularly early in the season, accounting for up to 85% of predators in soybean fields (Rutledge *et al*. 2004; Desneux *et al*. 2006). *O. insidiosus* can significantly suppress soybean aphid population growth (Rutledge & O'Neil 2005; Desneux *et al*. 2006); increases in *O. insidiosus* populations are also correlated to reductions in the densities of soybean aphids (Desneux *et al*. 2006). The impact of *O. insidiosus* can be particularly strong early in the season, when soybean aphids are colonizing fields and have not yet reached outbreak levels. However, little is known with regard to the strength of specific trophic connections within these food webs. Later in the season, if aphid densities increase, the predator complex can be enriched by other generalists, with *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) comprising > 90% of all coccinellids found (Rutledge *et al*. 2004; R.J.O., unpublished data). Given that coccinellids respond to higher aphid densities than other predators (Rutledge *et al*. 2004), they often appear when soybean aphid densities are peaking and near the time when soybean aphids produce autumn migrants.

Despite the density and diversity of natural enemies within soybean systems of the US Midwest, effective biological control can be compromised through predators switching to alternative prey (Koss & Snyder 2005; Prasad & Snyder 2006) and/or intraguild predation (Prasad & Snyder 2006; Gardiner & Landis 2007). To evaluate the role of alternative prey and intraguild predation, trophic linkages among predators and prey need to be defined. In recent years, molecular techniques have become increasingly important for elucidating interaction pathways and trophic structure of terrestrial food webs (Symondson 2002; Sheppard & Harwood 2005). These nonmanipulative approaches offer a valuable opportunity for studying the linkages between generalist predators and their prey, the structure of complex food webs, and whether trophic cascades and intraguild predation dampen the magnitude of population fluctuations within these systems. Whereas monoclonal antibodies are often advantageous in enabling the mass-screening of communities of arthropod predators for single, or relatively few, target prey (e.g. Hagler & Naranjo 1994, 2005; Symondson *et al*. 1996; Harwood *et al*. 2004, 2007), DNA-based gut-content analysis can allow rapid screening against a multitude of different prey likely to have been encountered in the field (e.g. Agust' *et al*. 2003; Harper *et al*. 2005; de León *et al*. 2006; Read *et al*. 2006; Juen & Traugott 2007). Furthermore, both approaches to gut-content analysis of predation can be of value in studying trophic connections by small predators in the field, without the interpretative difficulties associated with direct observations and the inability to discern predation upon many different, often cryptic, groups of prey.

In this study, we examine multiple trophic connections between the dominant native generalist predator, *O. insidiosus* (Desneux *et al*. 2006), and three potential prey items: specifically, the exotic pest *A. glycines*, a key species of alternative prey, *Neohydatothrips variabilis* (Beach) (Thysanoptera: Thripidae), and the exotic late-season aphid predator *H. axyridis*. These represent the most abundant arthropod fauna of soybean fields in Indiana (H.S.Y. and R.J.O., unpublished data), and *O. insidiosus* population dynamics closely follow those of thrips in US soybean fields (Isenhour & Marston 1981). By using field survey data and molecular gut-content analyses, we test the hypothesis that *O. insidiosus* will exhibit high levels of predation on soybean aphids early in the season. Finally, we examine if *O. insidiosus* attack eggs and larvae of *H. axyridis* in soybean fields, potentially limiting densities of these important late-season predators. Elucidating these trophic linkages is imperative if conservation biological control by native natural enemies is to be fully realized in successful integrated pest management programmes.

Materials and methods

DNA extraction and PCR analysis

Insects were collected from soybean fields at the Purdue University Agronomy Center for Research and Education (PUACRE; West Lafayette, Indiana, USA), and laboratory colonies of *Orius insidiosus*, *Aphis glycines*, *Neohydatothrips variabilis* and *Harmonia axyridis* were established under

Table 1 Primer sequences and GenBank Accession numbers. Values in the 'Size' column indicate size of amplicon in bp

Species	Primer sequence	Size	GenBank Accession no.
Orius insidiosus	F: ACACATTATTAGAAAAGAAAGAGGA R: TAAATAGAAATACGAATCCTAATG	281	EF467230
Aphis glycines	F: TTGTACAATCTTAAATATAATACCCA R: AAGAATAGGATCTCCCCCAC	255	EF467229
Neohydatothrips variabilis	F: GCATACTTTACATCTGCCACTA R: TTCCTGTCAATCCTCCTAATG	160	EF523586
Harmonia axyridis	F: AAAAAAATTGCCTTTGGTTCTT R: AATTGTAAATAAAAATAAAAATCCCA	261	EF192083*

*Optimal primer pairs were determined from sequences of *H. axyridis* collected from agroecosystems in North America, which were 100% identical, where they overlapped, to the published *H. axyridis* GenBank COI sequence (EF192083) for the 5′ 439 bp region, and 99.6% identical to AF515054 from bp 433–1257 due to an a/g substitution and an insertion in the sequenced individual (D.L.R., unpublished data).

controlled conditions of 22 ± 1 °C, 16 : 8 L:D cycle and 65 ± 5% RH. Prior to sequencing, insects were starved and preserved in 95% ethanol.

All samples were partially homogenized in 0.5 mL mortar-and-pestle microcentrifuge tubes in 100 μL of high salt extraction buffer (Aljanabi & Martinez 1997) supplemented with SDS to 2% and Proteinase K to 400 μg/mL. Samples were digested overnight at 60 °C. DNA precipitates were resuspended in 100 μL of 0.1× TE, pH 8.0. Preliminary polymerase chain reactions (PCRs) (30 μL) for nucleotide sequencing of cytochrome oxidase I (COI) utilized the following sets of primers: C1-J-1751 with C1-N-2191, C1-J-2195 with C1-N-2183, C1-J-2183 with TL2-N-3014 (Simon *et al*. 1994), and C1-J- 1751 'SPID' (Hedin & Maddison 2001) with C1-N-2776 per Promega's protocol. The following modifications were incorporated in the PCR: the addition of 0.1 μL of 20 μg/mL RNAseA (Gibco BRL), 1% polyvinylpurrolidone (Fisher Scientific) and 0.2% BSA (Sigma-Aldrich) to the cocktail (Xin *et al*. 2003) after the primers and before the *Taq* polymerase. PCR optimization utilized an initial denaturation for 3 min at 94.5 °C, followed by 40 cycles of 45 s at 94.5 °C, 1 min at 37 °C, and 2 min at 72 °C; 5 min at 72 °C completed the program. Reaction success was confirmed by electrophoresis of 6 μL of the PCR/Stop reaction in 1.5% agarose (Promega) in 0.5 × TAE (Promega). For those reactions that yielded strong PCR bands of expected size, the remainder of the reaction was loaded and the fragments for sequencing were excised from 1.5% NuSieve agarose (Cambrex Bio-Science) in 1× TAE with a final EDTA concentration of 0.1 mM. Sequencing of Indiana populations of *A. glycines* (n = 2), *O. insidiosus* (n = 2) and *N. variabilis* (n = 2) was undertaken using BigDye terminator version 3.1 kits on an ABI3100 sequencer (Applied Biosystems). Overlapping sequences were edited, assembled, aligned and the design of primers undertaken with Lasergene (DNAStar) and tested extensively with multiple replicates from soybean fields in Indiana. The haplotype sequencing of *H. axyridis* was undertaken on 24 specimens from Kentucky and 19 specimens from Maryland; all were identical for the overlapping (440 bp) region and reactivity confirmed by screening vs. populations from Indiana.

Predator- (*O. insidiosus*) and prey- (*N. variabilis* and *H. axyridis*) specific reactions were done as above except that the total reaction volume was 25 μL, only 35 cycles were performed, and the annealing temperature was 53 °C. In contrast, prey-specific reactions to *A. glycines* were modified such that the total reaction volume was 25 μL, and 45 cycles were performed with an annealing temperature of 62 °C. The selection of 45 cycles was done because a lower number of cycles (40) were not sufficient to amplify *A. glycines* as either a meal or from a single individual prepared and resuspended in the same volumes. Electrophoresis was performed in 2.25% agarose (Promega) in 0.5× TAE. Primer sequences, expected amplicon sizes and GenBank Accession numbers are given in Table 1.

The PCR assay was also screened for cross-reactivity with a diverse range of nontarget material (Table 2). Particular focus was given to closely related and nontarget species found in soybean crops to confirm false-positive reactivity would not occur when field-collected samples were screened against species-specific primer pairs.

Feeding trials

Three predator-prey feeding combinations were performed to determine detection limits of target DNA (*A. glycines*, *N. variabilis* or *H. axyridis*) following consumption. Prior to feeding trials, *O. insidiosus* colonies were established as above under controlled conditions of 22 ± 1 °C, 16:8 L:D

Table 2 Species tested for cross-reactivity against primer pairs

Order	Family	Invertebrates tested
Acari	Acaridae	*Tyrophagus putrescentiae* (Schrank)
	Tarsonemidae	*Polyphagotarsonemus latus* (Banks)
	Tetranychidae	*Tetranychus urticae* Koch
Araneae	Anyphaenidae	*Hibana futilis* (Banks)
	Clubionidae	*Clubiona kiowa* Gertsch
	Linyphiidae	*Florinda coccinea* (Hentz), *Frontinella pyramitella* (Walckenaer), *Grammonota texana* (Banks), *Meioneta unimaculata* (Banks)
	Lycosidae	*Pardosa milvina* (Hentz), *Rabidosa rabida* (Walckenaer)
	Salticidae	*Phidippus audax* (Hentz)
	Tetragnathidae	*Leucauge venusta* (Walckenaer)
	Theridiidae	*Achaearanea tepidiorum* (C.L. Koch)
Coleoptera	Cerambycidae	*Tetraopes tetrophthalmus* (Forster)
	Chrysomelidae	*Acalymma vittatum* (F.), *Chrysochus auratus* (F.), *Diabrotica undecimpunctata howardi* Barber, *Epitrix cucumeris* (Harris), *Lema trilinea* White, *Leptinotarsa decemlineata* (Say), *Leptinotarsa haldemani* (Rogers), *Leptinotarsa juncta* Germar
	Coccinellidae	*Coleomegilla maculata* (De Geer), *Coccinella septempunctata* L., *Cycloneda munda* (Say), *Epilachna varivestis* Mulsant, *Harmonia axyridis* (Pallas)
	Carabidae	*Agonum octopunctatum* F., *Agonum palustre* Goulet, *Agonum punctiforme* Say, *Agonum striatopunctatum* Dejean, *Amara aenea* (De Geer), *Amara anthobia* Villa, *Amara cupreolata* Putzeys, *Amara familiaris* (Duftschmid), *Amara sinuosa* (Casey), *Anisodactylus sanctaecrusis* (F.), *Bembidion affine* Say, *Bembidion nigripes* (Kirby), *Bembidion rapidum* LeConte, *Bradycellus nr. insulus* Casey, *Chlaenius nemoralis* Say, *Clivina bipustulata* (F.), *Clivina impressifrons* LeConte, *Cyclotrachchelus seximpressus* (LeConte), *Elaphropus anceps* (LeConte), *Elaphropus incurvus* (Say), *Elaphropus xanthopus* (Dejean), *Harpalus fulgens* Csiki, *Harpalus herbivegus*, *Harpalus indianus* Csiki, *Harpalus pennsylvanicus* Dejean, *Lebia grandis* Hentz, *Poecilus chalcites* (Say), *Pterostichus melanarius* (Illiger), *Pterostichus permundus* (Say), *Scarites quadriceps* Chaudoir, *Scarites subterraneus* F., *Stenolophus conjunctus* Say, *Stenolophus dissimilis* (De Geer), *Stenolophus lecontei* Chaudoir, *Stenolophus ochropezus* (Say)
	Tenebrionidae	*Tenebrio molitor* L.
Diptera	Calliphoridae	*Calliphora vomitoria* (L.)
Hemiptera	Aleyrodidae	*Bemisia tabaci* (Gennadius)
	Anthocoridae	*Orius insidiosus* (Say)
	Aphididae	*Aphis glycines* Matsumura, *Rhopalosiphum maidis* (Fitch), *Rhopalosiphum padi* (L.), *Schizaphis graminum* (Rondani), *Sitobion avenae* (F.)
	Geocoridae	*Geocoris punctipes* (Say)
	Nabidae	*Nabis alternatus* Parshley
	Pentatomidae	*Euschistus servus euschistoides* (Vollenhoven), *Oebalus pugnax* (F.), *Perillus bioculatus* (F.), *Podisus maculiventris* (Say)
Lepidoptera	Pieridae	*Pieris rapae* (L.)
	Plutellidae	*Plutella xylostella* (L.)
	Pyralidae	*Crocidolomia pavonana* (F.), *Galleria mellonella* (L.), *Ostrinia nubilalis* Hübner
Thysanoptera	Thripidae	*Echinothrips americanus* Morgan, *Frankliniella occidentalis* (Pergande), *Neohydatothrips variabilis* (Beach)

cycle and 65 ± 5% RH. Colonies were allowed to feed *ad libitum* on eggs of *Ephestia kuehniella* Keller (Lepidoptera: Pyralidae) prior to experiments, when approximately 200 individual predators were isolated and transferred into individual glass vials for 24 h starvation. *O. insidiosus* were given access to a moistened cotton ball to provide necessary humidity and moisture for survival.

After starvation, each *O. insidiosus* was transferred into a clear gelatin capsule (size 000; Capsuline) and fed a single unit of target prey (one *A. glycines* nymph, one *N. variabilis* adult, or one *H. axyridis* egg). Each *O. insidiosus* was observed to feed over a 2-h period, and the time at which they started and stopped feeding was recorded. At the end of the feeding period (t = 0 h), 10 *O. insidiosus* were frozen before being placed in 95% ethanol. All remaining predators were transferred into clean glass vials, maintained under controlled conditions (22 ± 1 °C, 16:8 L:D cycle and 65 ± 5% RH) and provided an *ad libitum* supply of alternative, nontarget 'chaser-prey' with a moistened cotton ball. The chaser prey were *N. variabilis* nymphs (for predators fed *A. glycines*), *H. axyridis* eggs (for predators fed *N. variabilis*) or *A. glycines* nymphs (for predators fed *H. axyridis*). Chaser meals were provided to simulate normal feeding rates and eliminate adverse effects of starvation on digestion rate and DNA detectability (Greenstone & Hunt 1993; Chen *et al*. 2000). Samples were then frozen and transferred into 95% ethanol at 4, 8, 12, 16, 20, 24 and 36 h after feeding (*A. glycines* treatment), 4, 8, 12, 16 and 24 h after feeding (*N. variabilis* treatment), and 1, 2, 3, 4, 8 and 12 h after feeding (*H. axyridis* treatment). These time periods are based upon pilot studies determining maximum detection periods for each predator-prey treatment (D.L.R. and J.D.H., unpublished data). For each time period and treatment, we used n = 10 predators. Following collection of *O. insidiosus* from all time periods and treatments, DNA was extracted from whole-body macerates as above and predators probed by PCR for DNA of both the desired target prey and the chaser meal.

Field sampling

Orius insidiosus were collected from early June until late August 2005 in an 11.9-ha soybean field (Asgrow 3602RR soybean, Monsanto Company, St Louis, Missouri, USA) at the Purdue University Agronomy Center for Research and Education, Tippecanoe County, Indiana, USA. Crops were grown in 76.2-cm rows, and raised according to standard agronomic practices for soybean production in Indiana with no application of pesticides. All samplings were undertaken in four control plots in the field with large buffer zones from adjacent crops of different management practices at the Purdue University Agronomy Center. Given that whole-body macerates were used to assess predation (as opposed to gut-extraction techniques), predators were collected individually by aspirator to avoid surface-level contamination with foreign DNA. Immediately following the collection of approximately 20 *O. insidiosus* per week, each predator was transferred into 1.5 mL microcentrifuge tubes and placed on ice before being transferred to a –80 °C freezer. Whole bodies were homogenized as above and screened for prey (*A. glycines*, *N. variabilis* and *H. axyridis*) and predator (*O. insidiosus*) DNA. Screening against *O. insidiosus* primers ensured no false-negative results and confirmed successful extraction of DNA from the samples.

In parallel with the collection of *O. insidiosus* for molecular gut-content analysis, predator and prey populations were sampled weekly to enable correlative analysis between prey availability and prey consumption. Insect surveys were conducted using destructive whole-plant counts (n = 30 per week) to estimate densities of *A. glycines*, *N. variabilis* and coccinellid egg masses; soybean plants were carefully uprooted, visually inspected and all prey species on the plants recorded. Sweep net sampling was also undertaken to determine densities of *H. axyridis* larvae (n = 8 sweep samples per week, each sweep sample consisting of 25 strokes).

Results

Primer specificity

The primers designed to detect predation upon *Aphis glycines*, *Neohydatothrips variabilis* and *Harmonia axyridis* by *Orius insidiosus* were screened for cross-reactivity against all nontarget prey items from soybeans and closely related organisms. These included aphids [*Schizaphis graminum* (Rondani), *Rhopalosiphum padi* (L), *Sitobion avenae* (F) and *Rhopalosiphum maidis* (Fitch)], thrips (*Frankliniella occidentalis* (Pergande) and *Neohydatothrips tiliae* (Hood)), carabids, coccinellids, pentatomids and arachnids (full list given in Table 2). In all instances, primers against *A. glycines*, *N. variabilis* and *H. axyridis* were specific to the desired organism, eliciting no amplification of DNA from any of the nontarget species screened. An example of an agarose gel of PCR-amplified DNA using the *A. glycines* primers screened vs. a selection of nontarget organisms is presented in Fig. 1.

Detection of prey DNA

The specific primers (Table 1) were used to screen *O. insidiosus* from laboratory feeding trials, in order to calculate DNA detection success following consumption of target prey (e.g. Fig. 2). Highly significant correlations were calculated for the percent of *O. insidiosus* screening positive for prey DNA and time since feeding (Fig. 3). In order to determine the decline in DNA detection success, we fit the data to the regression model of best fit as opposed

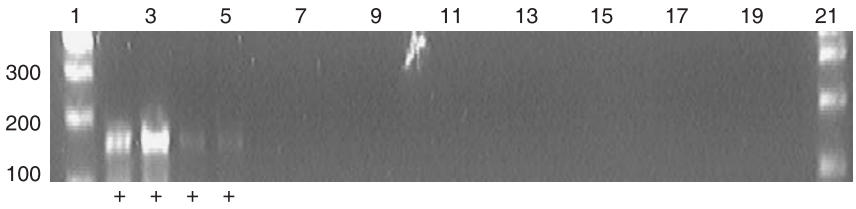

Fig. 1 Agarose gel of PCR products using *Aphis glycines* primers (Table 1) against target and select nontarget organisms. Lanes 1 and 21, 100 bp ladder; lanes 2 and 3, *A. glycines*; lanes 4 and 5, *Orius insidiosus* fed *A. glycines* (t = 0 h); lane 6, *O. insidiosus* fed *Neohydatothrips variabilis*; lane 7, *Echinothrips americanus*; lane 8, *Tetranychus urticae*; lanes 9 and 10, *Frankliniella occidentalis*; lanes 11 and 12, *Schizaphis graminum*; lanes 13–15, *Rhopalosiphum padi*; lanes 16 and 17, *Sitobion avenae*; lanes 18 and 19, *Rhopaloiphum maidis*; lane 20, no-DNA control. + signifies positive reaction.

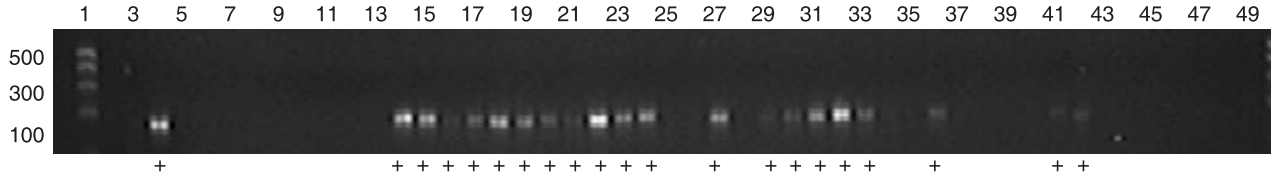

Fig. 2 Agarose gel of PCR products using *Neohydatothrips variabilis* primers (Table 1) to determine detection period of prey DNA following consumption by *Orius insidiosus*. Lane 1, 100 bp ladder; lane 2, *Orius insidiosus* fed *Harmonia axyridis*; lane 3, *O. insidiosus* fed *Aphis glycines*; lane 4, *O. insidiosus* fed *N. variabilis*; lines 5 and 6, *Echinothrips americanus*; lane 7, *Tetranychus urticae*; lane 8, *Frankliniella occidentalis*; lane 9, *Schizaphis graminum*; lane 10, *Rhopalosiphum padi*; lane 11, *Sitobion avenae*; lane 12, *Rhopalosiphum maidis*; lane 13, *Aphis glycines*; lanes 14–22, *O. insidiosus* fed *N. variabilis* (t = 0 h); lanes 23–32, *O. insidiosus* fed *N. variabilis* (t = 4 h); lanes 33–42, *O. insidiosus* fed *N. variabilis* (t = 8 h); lanes 43–49, *O. insidiosus* fed *N. variabilis*; lane 50, no-DNA control. + signifies positive reaction.

to an exponential decay model (Payton *et al*. 2003) given that some exponential regressions were not significant. The ranking of detection times for DNA of the three species within the guts of *O. insidiosus* at 22 °C is *A. glycines* > *N. variabilis* > *H. axyridis* within the guts of *O. insidiosus* at 22 °C. All predators were also screened for 'chaser' prey. At t = 0 h, no reactivity to chaser prey was observed while at subsequent time periods, all predators were positive for chaser meals (with the exception of one *O. insidiosus* at t = 8 h screening negative due to nonfeeding on chaser prey).

Analysis of field-collected predators

All predators were screened vs. the *O. insidiosus* primers to ensure successful DNA extraction; all predators in both field and laboratory trials screened positive. After an initial lag phase, *A. glycines* numbers increased exponentially (Fig. 4a). The proportion of *O. insidiosus* testing positive for *A. glycines* DNA (y) was highly correlated to aphid density (x) (arcsine square-root transformed $y = 3.71 \log x + 20.9$, $r^2 = 0.70$, $P < 0.001$, $n = 220$ *O. insidiosus*). There was evidence of significant early- season soybean aphid predation before aphid numbers increased (Fig. 4b). During collection dates of 23 June and 1 July, when mean aphid densities per plant (± SE) were 0.43 ± 0.27 and 0.41 ± 0.21, respectively, 13.3% and 6.3% of *O. insidiosus* tested positive for *A. glycines* DNA. Although predation upon *N. variabilis* was greater than would be predicted by their very low availability (Fig. 5), there was no relationship between

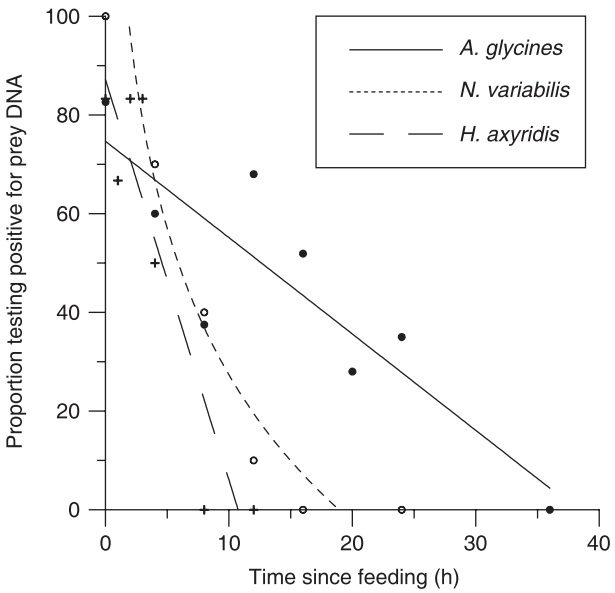

Fig. 3 Detection of DNA of (a) *Aphis glycines* (●) (b) *Neohydatothrips variabilis* (○) and (c) *Harmonia axyridis* (+) following consumption by *Orius insidiosus*. Regression equations: *A. glycines*: $y = -1.95x + 74.66$, $r^2 = 0.77$; *N. variabilis*: $y = -42.86 \ln(x) + 126.01$, $r^2 = 0.93$; *H. axyridis*: $y = -8.11x + 87.15$, $r^2 = 0.84$.

N. variabilis density and the mean percentage testing positive for soybean thrips DNA ($r^2 = 0.004$, $P = 0.852$). Mean weekly population densities of *N. variabilis* peaked at just 2.0% of those of *A. glycines*, while the greatest weekly

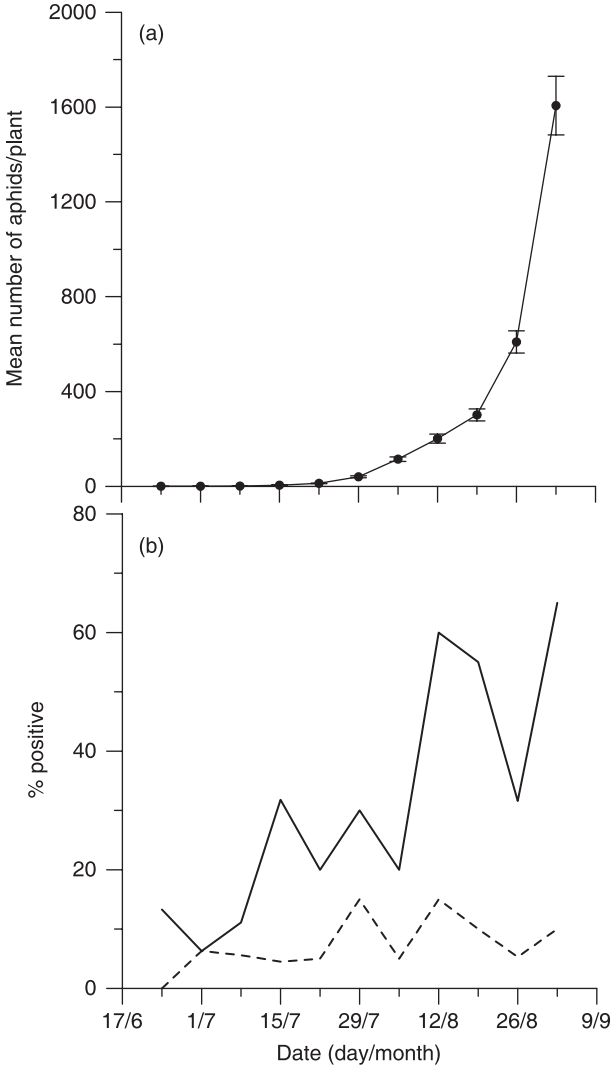

Fig. 4 (a) Mean number (± SE) of *Aphis glycines* captured on individual soybean plants during 2005; (b) the percentage of *Orius insidiosus* screening positive for *A. glycines* DNA on these sampling dates (solid line) and the percentage of *O. insidiosus* containing DNA for both *A. glycines* and *Neohydatothrips variabilis* (dashed line).

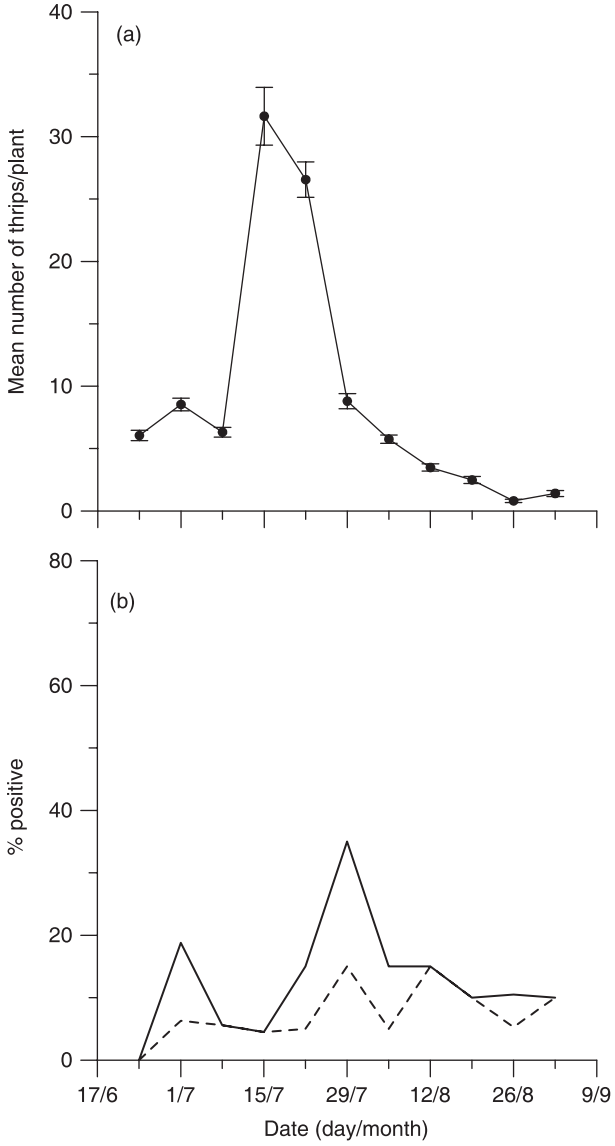

Fig. 5 (a) Mean number (± SE) of *Neohydatothrips variabilis* captured on individual soybean plants during 2005; (b) the percentage of *Orius insidiosus* screening positive for *N. variabilis* DNA on these sampling dates (solid line) and the percentage of *O. insidiosus* containing DNA for both *N. variabilis* and *Aphis glycines* (dashed line).

proportion of predators screening positive for aphid DNA (65%) was less than twice that of the greatest weekly proportion screening positive for thrips DNA (35%). However, the peak in thrips density during the sampling weeks of 15 July and 22 July (mean number per plant = 31.6 ± 2.3 and 26.6 ± 1.4, respectively; Fig. 5a) corresponded to increased feeding activity by *O. insidiosus* 1 week later (Fig. 5b).

Although *O. insidiosus* were screened for *H. axyridis* DNA, no predators screened positive for *H. axyridis* DNA despite the presence, albeit at lower densities than soybean aphids or soybean thrips, of egg masses and larvae in soybean fields during 2005 (Fig. 6).

Discussion

Primer design and PCR optimization

The application of molecular tools to study ecological interactions in the field relies on the specificity and sensitivity of the PCR primer pairs being developed. The primers for all prey items developed here were capable of detecting the DNA of *Aphis glycines* (bands produced at 194 bp), *Neohydatothrips variabilis* (159 bp) and *Harmonia axyridis* (261 bp). These primers also showed the appropriate levels of specificity when screened against

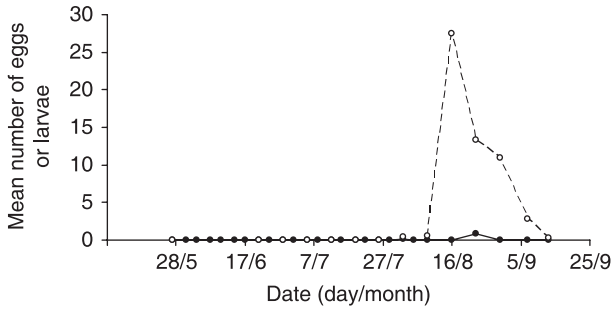

Fig. 6 Mean number of all coccinellid eggs (solid line, closed circle) and larval *Harmonia axyridis* (dashed line, open circle) in soybean fields during 2005. Egg numbers were determined from whole plant counts; larvae were average counts per sweep net sample of soybean fields.

nontarget prey from soybean fields and closely related organisms.

An additional consideration, prior to utilizing molecular markers to track trophic connections in the field, is the determination of the decline in target DNA detection success following prey consumption by the predator. The availability of 'chaser' prey is likely to increase detection periods because starvation can increase the rate of digestion of gut contents (Symondson & Liddell 1995). Chaser prey was used to simulate field conditions, in which nontarget food resources were available throughout the season, rather than an artificially simulated period of starvation. Detection limits of prey DNA varied significantly, a trait common among predator-prey feeding trials (Greenstone *et al*. 2007), thus requiring careful consideration during subsequent interpretation of field data. Furthermore, a rise in temperature can increase the rate of prey digestion, thus reducing detection periods for target material in predator guts (Hagler & Naranjo 1997; Hoogendoorn & Heimpel 2001). However, in the context of food-web studies, gut-content analysis undoubtedly offers the valuable attribute of being capable of discerning very specific trophic linkages occurring under natural field conditions, thereby allowing interactions to occur undisturbed between predators and prey in ecosystems. Other complications remain in that quantification of predation is not possible and occasional food-chain errors can occur due to secondary predation (Harwood *et al*. 2001; Sheppard *et al*. 2005) and scavenging (Calder *et al*. 2005; Foltan *et al*. 2005; Juen & Traugott 2005). Despite these limitations, which need careful consideration especially in the context of studies indicating the lack of differences between detection of prey DNA following consumption of live vs. scavenged food (Foltan *et al*. 2005; Juen & Traugott 2005), the likelihood of these errors is negligible due to the scarcity of dead prey within these soybean agroecosystems (H.S.Y., N.D. and R.J.O., unpublished data). Gut-content analyses can therefore be considered as providing reliable information pertaining to trophic connections in these highly complex and cryptic food webs.

Field analysis of predation: consumption of an invasive pest

In the soybean system examined, clear evidence was gathered to suggest that the *A. glycines* → *Orius insidiosus* trophic pathway was an important one, with 32% of predators screening positive for soybean aphid DNA. Such high levels of predation, coupled with the moderate DNA detection periods (Fig. 3), implicate *O. insidiosus* as a significant biological control agent of these invasive pests in Indiana. Although aphid populations sometimes crash due to fungal epizootics (Ragsdale *et al*. 2004), the absence of parasitoids and pathogens from many soybean fields (Rutledge *et al*. 2004) makes these predators particularly important in soybean aphid control.

Broadly, the role of generalist predators such as *O. insidiosus* in prey dynamics can be categorized as acting to (i) prevent outbreaks by targeting pests early in their population build-up, or (ii) reducing prey densities after they have achieved high densities late in the season (Murdoch *et al*. 1985). In the context of aphid populations, which tend to exhibit exponential growth as the season progresses (Fig. 4a), the latter is unlikely because generalist predators exhibit insufficient selectivity towards pests and consume a diverse range of arthropods. However, in order to prevent, or at least delay, the onset of exponential population growth, predators must be present early in the season and at sufficient densities to impact pests when they are invading the crop (Ehler & Miller 1978). We found *O. insidiosus* to be a very important early-season predator of *A. glycines*, with significant numbers of the population feeding on these pests when densities were extremely low (< 1 pest per plant). *O. insidiosus* clearly targets *A. glycines* at low densities although the specific mechanisms driving aphid predation are unclear.

Field analysis of predation: consumption of alternative prey

Neohydatothrips variabilis were an important alternative food for *O. insidiosus*. Despite the low densities throughout the year (Fig. 5a) and shorter DNA half-lives (Fig. 3), 12.9% of predators screened positive for target DNA, emphasizing the importance of this nonpest food resource. However, unlike the significant correlation between *A. glycines* density and percentage of *O. insidiosus* positive for aphid DNA ($r^2 = 0.70$), no relationship was documented between thrips availability and consumption ($r^2 = 0.004$). The molecular tracking of predation also revealed a 1–2 week lag in any increases in soybean thrips predation after densities showed

moderate increases in mid-July. Although cumulative predation events could lead to a build-up of prey material in predator guts over time in systems with extended detection limits (Sheppard & Harwood 2005), the relatively short DNA detection periods provided an accurate model for recent predation events in the field. Interestingly, of those predators screening positive for *N. variabilis*, 59.3% also contained DNA of *A. glycines* in their guts (compared to 23.5% of those predators screening positive for *A. glycines* which also contained *N. variabilis* DNA). This trend towards diversifying their diet with aphids could enhance the role of *O. insidiosus* as an important biological control agent of *A. glycines*.

Field analysis of predation: consumption of an intraguild predator

Intraguild predation can also play an important role in the dynamics of predation by natural enemies and their role in biological control (Rosenheim *et al*. 1995). While intraguild predation is common in agricultural systems (Lucas *et al*. 1998; Müller & Brodeur 2002), especially among coccinellids (e.g. Obrycki *et al*. 1998; Yasuda & Kimura 2001), hemipteran predators typically coexist with few examples of intraguild predation between them (Wheeler 1977; Neuenschwander *et al*. 1987). Although *O. insidiosus* will feed on coccinellid eggs in the laboratory, no field-caught *O. insidiosus* contained *H. axyridis* DNA in their guts. Despite the rapid decline in DNA detection success of *H. axyridis* DNA in *O. insidiosus* (Fig. 3), and lower population densities (Fig. 6) than soybean aphids and soybean thrips, one would expect to find at least a few positives if *H. axyridis* made up a portion of their diet. Levels of predation upon this intraguild predator therefore appear to be sufficiently low (or absent) to have little or no impact on biocontrol disruption through interactions between two important predators of *A. glycines*. This is especially evident given that 2005 was an outbreak year for *H. axyridis* (R.J.O., unpublished data); populations in other years are even lower thus signifying even less likelihood for intraguild predation in the field.

Conclusions

This research has provided conclusive evidence for the occurrence of high levels of early-season predation by a generalist predator upon an invasive pest of soybeans. Also, no negative predator–predator trophic linkages were documented, suggesting coexistence between *Orius insidiosus* and *Harmonia axyridis* in this soybean food web. Generalist predator food webs in soybeans are undoubtedly more complex than the specific linkages examined here, but this molecular analysis of *O. insidiosus* predation in soybeans implicates them as important natural enemies in the early season control of *Aphis glycines*. Further research is necessary to discern the strengths of all trophic linkages in this food web, particularly those with other alternative prey and intraguild predators that potentially disrupt levels of biological control.

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